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ACCESSORY CHROMOSOMES IN MAN.

MICHAEL F. GUYER,

UNIVERSITY OF CINCINNATI.

Since the publication of my papers on the spermatogenesis of the guinea and of the chicken respectively (Guyer '09*a*, '09*b*) in which was recorded the finding of a chromosome or chromosome complex comparable to the "odd," "accessory" or "X-element," described so frequently of late as occurring in a wide range of the Arthropoda, particularly the Tracheata, I have examined material from other vertebrates and can at present record its presence in the rat, its probable occurrence in the pigeon (although this will require some further corroboration), and its conspicuous occurrence in man. Inasmuch as the material for its study in the rat is in the hands of a student for further investigation, I shall confine myself in this paper to a description of the two accessory chromosomes as found in man, together with other features of human spermatogenesis.

For my studies on man I have been fortunate in being able to obtain exceptionally good material through the courtesy of my colleague, Dr. Paul. G Woolley. The subject from which the testicular material was secured was a negro thirty years of age who had died suddenly from the rupture of an aortic aneurism (Case no. 156143, Pathological Records, the Cincinnati City Hospital). A testis was removed within between an hour and an hour and a half after death while the body was still warm and slices were placed immediately into Gilson's and into Bouin's fluids.

The mounted sections were from five to twelve microns thick. Most of them were stained in Heidenhain's iron-hæmatoxylin

and counter-stained with Congo red or acid fuchsin, although Delafield's hæmatoxylin was used with some.

An abundance of cell divisions were found to have been in progress at the time of death. In a given field of the microscope, in a favorable region, it was not unusual to observe as high as six or seven cells in various phases of division. As many as five or six of such areas might exist in a single section, although it was more usual to find only one or two. The material was very uneven in that slides would be found in which section after section showed division stages, while in others divisions were scarce. These facts indicate that there were proliferating and resting zones in the testis. The stages found in most abundance were the metaphases and late prophases of the primary spermatocytes. It was a comparatively simple matter to find spindles on which the ordinary chromosomes were in metaphase with the two accessories, closely associated, well removed toward, or at, one pole (Figs. 6, 7, 8 and 9).

In the literature of the subject much confusion prevails regarding the number of chromosomes characteristic of man. There is wide disagreement in the counts of different observers and there seems to have been a great dearth of material showing division stages. Most of the enumerations are based on observations of from two to eighteen cells and these often in questionable stages of preservation. The great difficulty apparently has been to secure material which was sufficiently fresh or which was not diseased tissue that is notoriously irregular as regards karyokinetic phenomena.

As early as 1881 Flemming discussed mitosis in the case of man illustrating it with some six figures (Taf. 3, Figs. 11–16) of which Fig. 16 is from leucocytes of leucemic blood, the others, from the corneal epithelium of two different subjects from each of whom an eye had been removed because of affection of the bulbus. Although at this time he made no definite record of the number of chromosomes, his drawings show them to be considerably in excess of sixteen, the number later announced by Bardeleben ('92).

Writing several years later, however, in response to the 1892 paper of Bardeleben, Flemming ('97), from a reëxamination of

his old material, gives the number of chromosomes as twenty-four. He cites the papers of Hansemann ('91, '93) as the earliest attempts known to him to make a count of the chromosomes of man. But since Hansemann records eighteen in one case, twenty-four in another, and forty in a third, the latter apparently estimated from the spireme stage, and inasmuch as he himself admits that his count was very uncertain, concluding with the statement that, "die Zahl sicher höher als 24 sei," we may fairly disregard it, I think, in the light of modern technique. In this second paper Flemming ('97) states that his count is based on only four cell-divisions in which the chromosomes had just split preparatory to separation. His exact statement of his observations is as follows: "Es gelang das zwar bei keinen ganz sicher, aber bei zweien der vier darin enthaltenen Mitosen doch annähernd; es scheinen in beiden Fällen 24 Doppelchromosomen zu sein. Bei beiden sind es jedenfalls mehr als 22 und, wie ich sagen zu können glaube, weniger als 28; an einiger Stellen decken sie sich so, dasz eine exacte Zählung mir unmöglich wird." Flemming's material had been fixed in one sixth per cent. chromic acid and stained with safranin.

Bardeleben has published three papers ('92, '97, '98) on the spermatogenesis of mammals including man in which he comes to the conclusion that the number of chromosomes in the spermatogonia and spermatocytes of man are sixteen and eight respectively, and in his later papers he sets down four as the number that ultimately reaches the spermatids. That is, there is in successive divisions a reduction in numbers from sixteen to eight and then from eight to four. This is much the condition that I have found prevailing in birds (Guyer, '02, '09).

Wilcox ('00) studied sections from a testis which had been removed from a man fifty-four years old, in an operation for hernia. Although scrotal swelling had existed for a year previous to the operation the testis seemed to be normal in size and appearance. He reports that, "In my material the number seemed to be eighteen, the different counts resulting in figures ranging from fifteen to nineteen." He remarks however upon the striking absence of karyokinetic stages, so that his observations were based upon a very limited number of divisions. Because of

this lack of favorable stages he says: "The whole organ was, therefore, sectioned and in the great number of sections thus obtained, not more than twenty cells were found in mitotic condition."

Wilcox continues: "The few cases of mitosis observed were in spermatocytes of the first order. One could easily distinguish spermatogonia, spermatocytes of the first and second order, spermatids, and numerous nearly mature spermatozoa. The number of the latter to be seen was very large and precludes the assumption that the testis was functionally impaired by age or by hernia. In the opinion of the writer, this condition merely indicates that all the various processes in the spermatogenetic series are not necessarily to be observed as taking place at the same time. I can see no reason why there might not become established in the testis periods of cellular activity alternating with periods of cellular rest."

Unfortunately Wilcox gives no drawings with his paper nor does he state definitely whether he regards the eighteen chromosomes seen in the spermatocytes of the first order as the reduced number or not. He does remark, however, that, "in many cases they were plainly arranged in the tetrad or ring formation which has been observed in a pretty general variety of investigated species," consequently the inference would be that a synapsis had occurred and that one might expect to find in the neighborhood of thirty-six as the somatic number.

The latest investigation on the number of chromosomes in man with which I am acquainted is that of Duesberg ('06). He reviews the work of Hansemann, von Bardeleben, and Flemming and on the strength of his own observations concludes that Flemming's count of twenty-four is correct. The excessive number found by Hansemann he would account for on the basis of the abnormal increase in the number of chromosomes which is likely to occur in pathological tissues. In the case of Bardeleben he is inclined to believe that very thin sections (three microns) are responsible for the smallness of the count since he regards it as probable that part of the cell had been cut away.

The tissue upon which Duesberg worked had been fixed in Flemming's or in Hermann's fluid and stained by the iron-hæ-

matoxylin method. However, the number, twenty-four, which he records for man was not determined by direct count but was inferred from the fact that he found two or three clear cases of twelve chromosomes in the primary spermatocytes. That his finding of twelve in the primary spermatocytes was correct is borne out by my observations but he is not justified, in consequence, in stating that there must be twenty-four in spermatogonial or somatic cell-divisions. My material shows that two of the twelve chromosomes are the univalent accessories, and a clear count of favorable spermatogonial chromosomes reveals only twenty-two. This means in all probability that of the twelve chromosomes of the primary spermatocyte, ten are bivalent and two accessories. Although Duesberg examined the spermatogonial chromosomes he states that he was unable to count them exactly beyond determining that, contrary to the opinion of Von Bardeleben, there were clearly more than sixteen. He states further (p. 477) that, "Je n'ai pas pu les compter exactement, tant à cause de la petitesse des cellules que du nombre assez élevé des chromosomes, mais dans quelques cas favorables où leur numération a pu être entreprise, j'ai obtenu des résultats très voisins de 24, jamais supérieurs à ce nombre dans les cellules normales." And in conclusion he says: "Il résulte de là que le nombre des chromosomes est certainement à mon avis, de 12 dans les spermatocytes et par conséquent de 24 dans les spermatogonies et les cellules somatiques. C'est la confirmation de l'opinion de Flemming."

In the general scheme of the spermatogenesis of man there appears to be nothing unique. One can readily recognize the usual four generations of germ cells; viz., spermatogonia, primary spermatocytes (or spermatocytes of the first order), secondary spermatocytes (or spermatocytes of the second order), and lastly spermatids which transform directly into the spermatozoa. An abundance of easily identified Sertoli or nurse cells are in evidence. Occasional centrosomes were observed in suitably stained preparations but I have not pictured any in my drawings because the preparations from which the latter were made were all so strongly decolorized that the stain had evidently completely disappeared from any centrosomes which might have been present.

The matter of counting the spermatogonial chromosomes, it must be admitted, is one of great difficulty. In the late prophase or equatorial plate stage, the only time at which a count is possible, they lie for the most part in an irregular band around a central clearer area. In the vast majority of cases only a deeply stained mass of small contiguous or overlapping chromosomes is visible in this band and an accurate count is out of the question although one can frequently determine that there are over twenty. In several instances, however, in which the positions of the chromosomes and the degree of the staining were favorable, twenty-two distinct chromosomes, never more, were visible.

There is considerable range in size among the individual chromosomes of the spermatogonia as well as observable differences of form. Most of them were rod-like or oval in shape although some were more nearly spherical. In several though by no means all instances two chromosomes, closely associated, were seen lying at some distance away from the main band, out in the cytoplasm. Taking into account this isolation, the rounded shape of these chromosomes and their relative sizes, it seems very probable that they are the two accessory chromosomes which do not manifest their presence for a certainty until the next division. It will be observed that one is somewhat smaller than the other. This condition obtains also between the two chromatin nucleoli of subsequent stages as well as between the accessory chromosomes whereever they can be identified, and one is led in consequence to strongly suspect that they are all one and the same thing. This inference is all the more justifiable when the relation between the chromatin nucleoli and the accessory chromosomes in some of the lower forms is recalled.

Fig. 2 represents a nucleus of the primary spermatocyte in the spireme stage which shows the two chromatin nucleoli in question. In deeply stained specimens these nucleoli, especially the smaller one, are not always evident but in preparations stained by the iron-hæmatoxylin method and then almost entirely decolorized, even as regards the ordinary chromatin of the spireme they are usually conspicuously visible. It should be mentioned that occasionally other small nucleolus-like granules were observable but since there was no constancy in their presence, size or relationship, I have

felt justified in ignoring them in the present discussion. Fig. 3 represents the spireme in the contraction phase which is not very pronounced in man. It will be observred that the two characteristic chromatin nucleoli still persist.

The primary spermatocytes when ready for division, as has already been stated, reveal twelve chromosomes in late prophase or early metaphase (Figs. 4, 5). In Fig. 4 the two accessories are seen at a glance and the remaining chromosomes, judging from their increased size and changed form, are bivalent, representing the paired univalent chromosomes of the spermatogonium. That is, of the original twenty-two chromosomes twenty have paired to form the ten bivalents of the primary spermatocyte and two have remained unpaired as the accessory chromosomes. In Fig. 4 it is not evident just which two are the accessories although twelve chromosomes are present.

It is obvious from the the figures (Figs. 4–9) that there is considerable difference in the size of the various chromosomes of the primary spermatocyte. Although the attempt was made it was not found possible to always identify the individual chromosomes. They grade down in size from some three or four large ones to two or three small ones but the fluctuations in size, probably due for the most part to differences in the effects of fixation together with different degrees of extraction of the stain, were too great to render identification sure. In very strongly decolorized sections, especially when counterstained with Congo red, one large chromosome in particular frequently exhibited a tetrad-like formation, while the other large ones at times showed more or less definite indications of lobing. In some cases this was sufficiently marked to interfere with accurate counting. In a very few instances, so few I think as to be practically negligible, there appeared to be fourteen instead of the customary twelve chromosomes, but the extra chromosomes always took the form of a tiny pair which I am inclined to think had become split off from one of the ordinary tetrads or which had through some chance never entered into the proper tetrad formation. They were always united by linin-like strands to one or two of the larger chromosomes.

Figs. 6, 7, 8 and 9 show the two accessories in characteristic positions. Side by side, they always pass entire, considerably in advance of the divided ordinary chromosomes, toward one pole.

Of the ordinary daughter chromosomes of this first spermatocytic division, a pair of small elongated ones not infrequently are the first to emerge from the general equatorial mass as shown in Fig. 7. One is led to suspect that they may possibly be comparable to the small pair of chromosomes found so constantly in certain of the Tracheata although the evidence is not sufficiently decisive to make this an established fact.

It is inferred that the division of the primary spermatocyte is the reducing division, not simply because such a division ordinarily occurs at this stage, but from the fact that the chromosomes after divergence (Figs. 10, 11) when compared with corresponding divisions of the secondary spermatocytes are seen to resume more the elongate, rod-like appearance that characterizes the univalent spermatogonial chromosomes, and also because the accessory chromosomes pass over entire to one pole here while they are halved in the next division.

It is evident from the foregoing that as regards chromatin content the result of the division of the primary spermatocyte is the production of two dissimilar cells, one of which receives ten, the other, twelve chromosomes. Fig. 10 is a drawing of one end of a late anaphase of such a division showing twelve chromosomes (10 plus 2 accessory). Fig. 11, in which only ten chromosomes are visible, was drawn from what is probably the reverse end of a somewhat later anaphase than that shown in Fig. 10. It is just possible that it is a prophase of division in a secondary spermatocyte where univalent chromosomes come to the equator, but if so it is the exception rather than the rule, as the secondary spermatocytes ordinarily divide according to a different scheme. In any event the drawing serves to illustrate the fact that some daughter cells of the primary spermatocytes have twelve chromosomes and some only ten.

In places both primary and secondary spermatocytes were found dividing in the same field and one is led to conclude that either there was no intervening period of rest between the two divisions or that it was a very brief one. In other instances, however, undoubted resting stages of secondary spermatocyte nuclei were seen in abundance. Approximately half of them showed, under proper decolorization, two chromatin nucleoli of which one was somewhat smaller than the other.

While at the conclusion of the divisions of the primary spermatocytes ten and twelve chromosomes respectively were delivered to the pairs of daughter cells, nevertheless, when the latter as secondary spermatocytes become ready for division, half of them show five and the remainder seven chromosomes. A second pairing of the ordinary chromosomes has evidently occurred, so that there are five bivalent chromosomes in each type of cell and the additional two accessories in the one type. Fig. 12 is a drawing of two contiguous secondary spermatocytes; the one shows five bivalent chromosomes in late prophase, the other more than five chromosomes in metaphase. These two cells are undoubtedly the two daughter cells of the same primary spermatocyte. Fig. 13 shows one daughter cell containing a group of seven chromosomes and the other a late anaphase of division which shows at one end five chromosomes. The number of chromosomes at the opposite pole of the second cell should of course be five although because of the dense massing it could not be positively determined. Fig. 14 represents a late anaphase of division in a secondary spermatocyte which manifestly had had seven chromosomes in metaphase.

Both accessory chromosomes divide in this second spermatocytic division period so that each resulting spermatid receives seven chromosomes (Fig. 16). Fig. 15 represents an anaphase of division in a secondary spermatocyte showing still at the equator of the spindle a lagging chromatic mass. Such a condition was found in several instances and while I believe it to be the two accessory chromosomes which happened merely to be unfavorably placed for observation, I could not positively identify it as such. From the relative positions of the chromosomes as seen in Fig. 16 one would infer that the two sets of accessories were the last to have passed from the equator to the poles of the spindle. Moreover, such a lagging of the accessory in this division was observed in both the guinea and the chicken (Guyer, '09).

It should be mentioned that occasional division stages were visible which, judging from the smallness of the cell and the size and shape of the chromosomes, looked as if they might be secondary spermatocytes preparing to divide with the univalent type (ten or twelve) of chromosome. It is possible, for instance, that

Fig. 11 represents a prophase of the secondary rather than an anaphase of the primary division although I am inclined to think it is the latter. If such simple divisions do take place, however, they are certainly scarce in the material which I have examined so far.

From the foregoing evidence it is manifest that there are in all two distinct groups of spermatids equal in number; namely, those which have received five and those which have received seven chromosomes. These chromosomes soon lose their visible identity and the spermatids are apparently all alike except for the significant fact that approximately half of them, in such preparations as have been stained by the iron-hæmatoxylin method and then all but entirely decolorized show two chromatin nucleoli. It would seem probable that these nucleoli stand in direct genetic continuity with the two eccentric chromosomes seen in the spermatogonia and the two chromatin nucleoli and the accessory chromosomes of the spermatocytes. Fig. 18 represents two contiguous spermatids, one of which shows no nucleoli, the other, two. Comparison with Fig. 19 shows the relative conditions of size between the nucleoli of the spermatid and those of a primary spermatocyte.

As to the meaning of the second conjugation there seems to be at present no clew. I have commented on it briefly in a former paper ('09*a*, p. 509). It is not peculiar to man for I have observed it also in the pigeon ('02, '03), the guinea ('09*a*) and the rooster ('09*b*). Undoubtedly Bardeleben ('97, '98) still earlier saw the same phenomenon in man, for although my results do not agree numerically with his count of sixteen, eight and four respectively, evidently, from the relative proportions in his counts, he had come upon this second curious numerical reduction.

Assuming that the respective chromosomes are more or less qualitatively differentiated, such a numerical reduction, however, by no means necessarily implies that there has also been a second qualitative reduction. Aside from the improbability of such a reduction, the general appearance of the divided chromosomes would not warrant this interpretation; for instead of the elongated univalent type as seen in the spermatogonia or in anaphases of the divisions of spermatocytes of the first order, the daughter chromosomes here retain the rounded appearance and increased size that

is characteristic of the bivalent types (compare Figs. 1, 10 and 11, 13, 14, 15, 16 and 17). Thus while half of the spermatids receive five, and half seven chromosomes, in terms of univalence the numbers would in all probability be ten and twelve respectively.

Inasmuch as the spermatids transform directly into spermatozoa it follows that there must be be two classes of the latter differing with respect to whether they have or do not have the two accessory chromosomes. Thus the conditions in man appear to be very much the same that Wilson ('09) describes for *Syromastes*, one of the squash-bug family (Coreidæ).

Numerous examples of such dimorphism of the spermatozoa have been recorded in various invertebrates, particularly in insects, arachnids and myriapods, and it has been clearly demonstrated that eggs fertilized by spermatozoa which possess this accessory chromosome or chromosome group (there may be one, two, three or even four separate chromatic bodies, depending upon the species) develop into females, those fertilized by spermatozoa which do not possess it, develop into males. Hence the accessory has come to be regarded by some of our most careful and experienced workers as an actual sex determinant. In any event it is obviously associated with the determination of sex either as cause or effect. In the light of numerous recent researches both on plants and animals the idea has rapidly gained ground that sex arises not as was long believed, as a response of the developing organism to stimuli from without, but that under normal conditions at least, it is automatically determined by some internal physiological mechanism.

Inasmuch as this intricate matter has been repeatedly and exhaustively discussed pro and con during the past ten years, it is unnecessary for me to enter into a review of the subject anew. For the general reader who may not have kept in touch with the current literature of the subject, two excellent critiques are now available in the recent papers of Wilson ('09*a*) and Morgan ('10). In these papers one will also find thoroughgoing discussions of the subtle problem as to whether, assuming that the accessories are sex determinants, the matter of sex determination is to be regarded as a qualitative process effected by some inherent peculiarity of the accessory chromosome, or whether the relation of such a chro-

mosome or group of chromosomes to sex is merely a quantitative one, the female type resulting when a greater amount of active chromatin is present. Extensive bibliographies will be found in the recent papers of Wilson ('05, '06, '09), Payne ('09), Morse ('09) and Morgan ('10).

In conclusion I wish merely to point out that as regards accessory chromosomes, conditions prevail among vertebrates (guinea, chicken, rat, man, etc.) similar to those found among numerous Tracheata (and probably certain other invertebrates) where the accessories are undoubtedly associated in some way with the phenomena of sexuality. In *Syromastes* (Wilson, '09*b*), which seems to parallel most nearly the condition found in man, half of the spermatids were found to possess two more chromosomes than the remainder. It was predicted by Wilson that in consequence the somatic cells of the female of this species would show two more chromosomes than the somatic cells of the male. Later the facts were found to be in exact accord with his prediction, the somatic cells of the female containing twenty-four, of the male twenty-two chromosomes. Similar verifications have been made in other tracheate forms.

In the light of these facts we should expect the somatic cells of man to contain twenty-two, and of woman, twenty-four chromosomes. The tissues of the female have not yet been studied with this in mind. Flemming ('97) records the somatic number of chromosomes, determined from corneal cells, as twenty-four but unfortunately he does not record the sex of the subjects from which the material was obtained. If it were a female his count would bear out the interpretation given above.

Summary.

1. Twenty-two chromosomes differing considerably in size occur in all spermatogonia in which a definite count could be made. In a few instances two, apparently the two accessory chromosomes, were seen considerably to one side of the main mass of chromosomes, surrounded by a small clear court of cytoplasm.

2. Twelve chromosomes appear for division in the primary spermatocyte, of which ten are evidently bivalent and two accessories.

3. The two accessory chromosomes pass undivided to one pole of the spindle considerably in advance of the other chromosomes, with the result that half of the daughter cells in this division receive twelve, and half, only ten univalent chromosomes. This is evidently the reduction division.

4. The ten univalent chromosomes which passed to the one secondary spermatocyte unite again in pairs, at least in the majority of cases, to form five bivalent chromosomes which appear at the equator of the spindle when the cell is ready for division. The division here is presumably an equation and not a second reduction division, judging from the size, shape and general appearance of the resulting daughter chromosomes. Thus while each of the spermatids formed as a result of this division receive only five chomosomes, the latter are bivalent and equivalent to ten of the somatic or spermatogonial chromosomes. There is some slight evidence that the secondary spermatocytes may occasionally divide with these chromosomes in their original condition of univalence.

5. Ten of the twelve chromosomes which passed to the other pole of the spindle in the primary spermatocyte behave in precisely the same way as described in the last paragraph. The two accessory chromosomes come to the equator of the spindle in the secondary spermatocyte with the five bivalents thus making in all seven. Each accessory now divides so that the resulting spermatids each receive seven chromosomes; that is, five bivalent plus two accessory, or the equivalent of twelve univalent chromosomes.

6. In reality, then, of the total number of spermatids, half have in all probability received ten, and half, twelve (10 plus 2) univalent chromosomes. Inasmuch as the spermatids transform directly into spermatozoa, there must be two classes of the latter differing with respect to whether they have or do not have the two accessory chromosomes.

7. It is a significant fact that approximately half the resting spermatids when strongly decolorized after iron-hæmatoxylin staining, show two chromatin nucleoli and half do not. It seems probable that these nucleoli may correspond to the accessory chromosomes and are to be identified with the two

nucleoli of the primary spermatocyte and the two eccentric chromosomes seen in the spermatogonia.

8. It is probable that in man and certain other vertebrates, as in the insects, myriapods and arachnids, the accessory chromosomes are in some way associated with the determination of sex.

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Explanation of Plate I.

All of the drawings were made with the aid of a camera lucida; their magnification is approximately 1,550 diameters. While the chromosomes are represented as accurately as possible, no attempt has been made to show details of the achromatic structures beyond general appearances and relations. In several cases where the chromosomes were not in the same focal plane they have been drawn carefully in the most favorable plane and then corrected as much as possible from second more accurate drawings of such individual chromosomes as were not in focus in the first drawing.

Fig. 1. Late prophase of spermatogonial division showing twenty-two chromosomes. The two chromosomes lying to one side of the main zone of chromosomes are presumably the two accessories.

Fig. 2. Nucleus of primary spermatocyte showing spireme stage with two chromatin nucleoli.

Fig. 3. Nucleus of primary spermatocyte showing the contraction-phase of the nuclear contents, also two persisting nucleoli.

Fig. 4. Late prophase of division in a primary spermatocyte showing twelve chromosomes. The two lying to one side of the main group are the accessories.

Fig. 5. Late prophase of division in a primary spermatocyte showing twelve chromosomes. Here the two accessories are not readily identified.

Figs. 6, 7, 8, 9. Metaphases of divisions in primary spermatocytes showing the two accessories in characteristic positions passing early to the poles. Fig. 7 shows also two precociously diverging daughter chromosomes.

Fig. 10. One end of a late anaphase of division in a primary spermatocyte showing twelve chromosomes (10 plus 2 accessory).

Fig. 11. Probably one end of a late anaphase of division in a primary spermatocyte, showing ten chromosomes, the accessory chromosomes having gone to the opposite pole; possibly a prophase of division in a secondary spermatocyte in which the ten chromosomes have remained univalent.

Fig. 12. Two contiguous secondary spermatocytes of which one shows five bivalent chromosomes in late prophase, the other more than five chromosomes (probably five bivalent plus two accessory) in metaphase. These two cells are evidently the products of a division of a primary spermatocyte in which ten chromosomes passed to one pole and ten plus the two accessories to the other.

Fig. 13. Two contiguous secondary spermatocytes. One, having just divided, shows five chromosomes at one pole; the chromosomes at the other pole are so massed as to preclude counting although there should be five. The other secondary spermatocyte shows seven chromosomes in late prophase.

Fig. 14. Late anaphase of division in a secondary spermatocyte which has received the two accessory chromosomes, showing seven chromosomes in all.

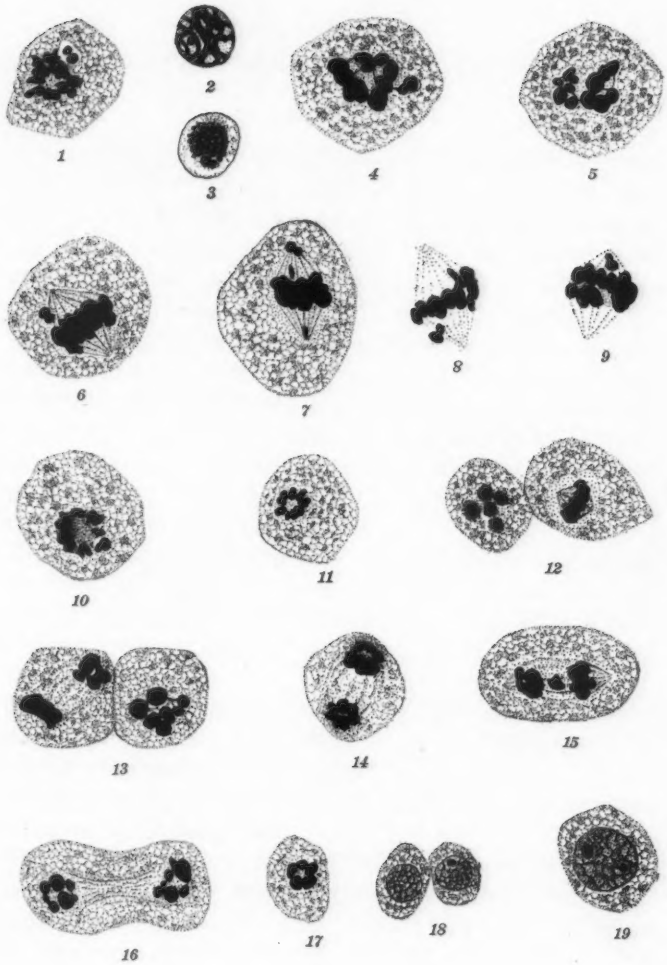
Fig. 15. Anaphase of division in a secondary spermatocyte showing still at the equator a lagging chromatic mass which is probably the two accessory chromosomes although it could not be positively identified as such.

FIG. 16. Late anaphase of division in a secondary spermatocyte which has received the two accessory chromosomes. Each of the latter divides as an independent chromosome at this time.

FIG. 17. One end of a late anaphase of a division in a secondary spermatocyte which had not received the accessory chromosomes.

FIG. 18. Two contiguous spermatids, one without chromatin nucleoli, the other with two. The spermatids in general are about equally divided into these two classes.

FIG. 19. Nucleus of a primary spermatocyte showing two chromatin nucleoli.



MICHAEL F. GUYER.

ON THE RIGHTING MOVEMENTS OF THE STARFISH.[1]

A. R. MOORE.

In an article entitled "The Behavior of the Starfish, *Asterias Forreri* de Lariol,"[2] Jennings discusses the movements of that animal in righting itself. He assumes that the starfish makes its movements in order to adapt itself to its environment and that therefore these movements are purposeful. From this assumption he concludes that "when the starfish is turned over on its dorsal surface locomotion is impossible, the finding and capture of food must stop; the delicate gills are pressed against the bottom, injuring them and impeding respiration; and displacements of the internal organs must occur that may be harmful to their proper functioning. We find, as might be anticipated, that there is a regulation of these bad effects by movement; the starfish turns again on its ventral surface."

A much simpler explanation of the righting movements has been given by Loeb.[3] He points out that the tube feet are positively stereotropic. Therefore the arms twist and turn until all of the tube feet are in a position to be in contact with a surface.

I have made observations on about thirty specimens of *Asterina miniata* and a like number of *Asterias ochracea*, with a view to determining the nature of the righting movements.

Let us look first at the causes, which Jennings has given us, for the starfish righting itself.

In regard to the first, viz., that locomotion is one of the ends which a starfish has in view in righting itself, I have found that very frequently the starfish crawls up the side of the aquarium and, upon reaching the surface of the water, thrusts out three or four arms dorsal side downward, their tube feet clinging to the surface film of the water. In such a position they remained

[1]From the Herzstein Research Laboratory, New Monterey, Cal.

[2]Jennings, *University of California Publications*, Zoology, Vol. 4, pp. 53–185.

[3]Loeb, "Comparative Physiology of the Brain," Chapter 3.

sometimes for more than an hour, although further locomotion was impossible, and no attempt at righting was made. In fact, the starfish often retained such a slight attachment to the wall of the aquarium that the surface film of the water could no longer support the weight of the animal, with the consequence that the latter fell to the bottom of the tank. In such cases the tube feet cling to the surface film of the water because the film acts as a solid surface; it cannot, however, bear the animal's weight. Romanes[1] speaks of these movements as follows: "On reaching the surface, the animal does not wish (!) to leave its native element . . . and neither does it wish (!) again to descend into the levels from which it has just ascended. It therefore begins to feel about for rocks or sea weeds at the surface, by crawling along the side of the tank and every now and then throwing back its uppermost ray or rays along the surface of the water to feel for any solid support that may be within reach." Romanes evidently was not familiar with surface tension. Had he known that the surface film of a liquid acts like a solid surface he would have been prevented from attributing intelligence to the starfish.

In order to see whether pressure on the gills might, as Jennings states, cause the starfish to turn over, I supported a glass plate in the aquarium, at a height just sufficient to press lightly on the dorsal side of a starfish moving over the floor of the tank. This was placed in the path of an approaching starfish. The latter did not change its direction when the plate was touched, but pursued its course, although the gills were pressed down. Furthermore, if a starfish is allowed to attach itself to a glass plate and is then suspended dorsal side downward so that it touches the bottom, its movements continue normally, although it could easily right itself if that were necessary. Clearly, then, pressure on the gills is not one of the factors which causes a starfish to right itself.

The displacements of the internal organs which, we are assured, "must occur" when the dorsal side is down, can only be due to gravity. I have frequently observed large numbers of starfish clinging, dorsal side downward, to overhanging ledges, feeding

[1]Romanes, "Jellyfish, Starfish and Sea Urchins," p. 268.

on barnacles and mollusks. Surely, "the displacements of the internal organs which *must* occur" when the dorsal side is downward, do not interfere in the least with the ingestion and digestion of food. Such "displacements" can, therefore, hardly be considered seriously as causes for the righting movements taking place.

We are forced to conclude, from the observations described, that, as Loeb has stated, the starfish ceases its efforts to right itself the moment all the tube feet can be brought into contact with a solid surface. Gravity plays no part in the righting movements.

The idea has been advanced by Loeb[1] that the mechanism of the righting movements is the result of coördinating and inhibiting impulses, which are transmitted to the various arms by the ventral nerve ring.

Six distinct methods of the righting reaction have been described by Jennings, but he has made no analysis of them on the basis of inhibiting and coördinating impulses. My observations agree with Loeb's assumption and give a rather simper explanation of the behavior of the starfish.

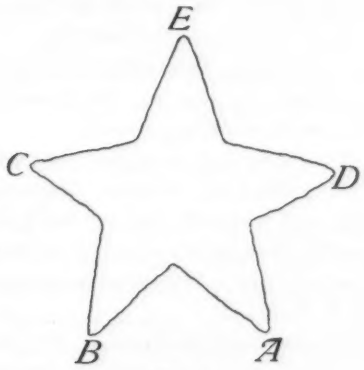


FIG. 1.

As a rule, when a starfish is placed upon its dorsal side, the arm whose tube feet first touch bottom determines the course of the righting. This arm begins at its distal end to twist the

[1]Loeb, "Comparative Physiology of the Brain," Chapter 3.

dorsal side upward, and as rapidly as the twisting is accomplished the tube feet secure a hold on the bottom. Next, if not simultaneously, the arm adjacent to the ventral side of the arm which is obtaining a hold, twists, so that the ventral surfaces of the two arms face each other, and secures itself in the same manner as the first. If *A* and *B* have in this way attached themselves to the bottom, inhibiting impulses are sent to *C* and *D*. The latter release themselves if they have already seized the bottom, rise ventrally, dragging *E* which either remains passive or bends dorsally, even catching the bottom with its tube feet in some cases. The righting is completed by *C*, *D* and *E* passing over *A* and *B* and attaching.

This simple and useful method of righting may be modified by (1) inequalities in the length of the arms, (2) injuries to certain of the arms, (3) any initial twist an arm may have due to its position before the animal was laid upon its dorsal side.

As to (1) short arms are more sluggish than ones of normal length, (2) injury to an arm inhibits the active twisting and seizing of the surface with the tube feet of this arm, (3) if an arm is partially twisted its tube feet reach the bottom more quickly than they otherwise would. As a result we have the following modifications of the normal method of righting.

1. If four arms are injured, their activity is inhibited and the righting is accomplished by the one uninjured arm. It may force an adjacent arm to coördinate weakly.

2. If *A* and *C* (Fig. 1) twist so as to face each other with their ventral surfaces, *B* receives two impulses, from opposite directions, to coördinate, and therefore does not twist either way but bends under dorsally, allowing *A* and *C* to accomplish what *A* and *B* did in the normal case. The same result may be brought about by injuring *B*, *D* and *E*.

3. Sometimes inhibitions are weak and *A*, *B*, *C* and *D* may all remain attached, *C* and *B* facing ventrally toward *A* and *D*. *E* alone is inhibited and the righting is accomplished by *A* and *B* walking backward under *C* and *D*.

I found, as Jennings noted, that in a few cases a starfish persistently refused to use a certain arm for initiating the righting movements. In most cases this was clearly due to an injury or

malformation of the inactive arm. According to the author cited, such a starfish could be "taught" to use the idle arm by giving the animal a large number of "lessons" (180 in one case) in which arms ordinarily active were prevented from taking hold by "stimulating their tube feet with a glass rod" whenever they attempted to attach themselves.

I was able to compel starfish of this sort to use the idle arm by injuring the active ones in the following ways: (1) Irritating the ventral groove of the arm by rubbing it with a glass rod, (2) treating the tips of the arm with a few drops of $n/10$ acid. Two or three applications a few minutes apart usually sufficed to render the arm inactive. In this way I was able to "teach" the starfish in one "lesson," spontaneously to use an arm previously inactive. The length of time the lesson was "remembered" depended upon the degree of the injury. It seems evident from this that Jennings' "lessons" consisted merely in inhibitions due to the injury caused by his irritating the tube feet of the active arms. But an inhibition caused by a single or persistent stimulation is not identical with the phenomena of memory manifested in the process of teaching.

Summary.

1. The righting movements of a starfish which has been placed on its dorsal side are due only to the positive stereotropism of the tube feet.

2. An injury to an arm inhibits its being used for the initiation of righting movements.

3. A starfish cannot be taught to use an arm which is ordinarily passive, but by injuring the other four arms these can be prevented from initiating righting movements and the fifth arm then initiates these movements.

I wish to express my sincere thanks to Professor Loeb for his helpful suggestions and criticism.

A SIMPLE COOLER FOR USE WITH THE MICROTOME.

CASWELL GRAVE AND OTTO C. GLASER.

The microtomist's ability to prepare thin paraffin sections, depends, among other things, on the hardness of his imbedding medium, and this, in turn, on the temperature of the laboratory. Usually this circumstance offers no insurmountable difficulties, but there are times and places when this is not true. To meet such conditions several devices have been suggested and used by various investigators, but we know of none so simple, or as little likely to make difficulties, as the one about to be described.

The apparatus, which is shown, set up for action, in Fig. 1, is essentially a hollow truncated pyramid, open at both ends, and suspended in an inverted position from a standard, so adjusted that the lower end of the shoot is at a convenient distance above the knife. At the upper end of the inverted pyramid, and surrounded by it, is a tray whose dimensions are less than those of the base of the shoot. This tray is filled with crushed ice, and from one corner of it a drain leads the water to the escape from the lower end of the air-channel. At that point a rubber tube connects the pipe with a suitable receptacle.

The cooler is easily set up, interferes in nowise with the operator, and is thoroughly effective. When the air of the room strikes the melting ice in the tray, it is chilled and immediately falls between the tray and the walls of the pyramid. In this way a constant stream of cold air pours from the lower end of the shoot, and as this may be placed directly above the paraffin-block and knife-edge, both of these are cooled, and make it possible to cut sections very much thinner than the unmodified temperature of the room would allow.

The extent to which it is desirable to cool the paraffin and knife varies with each specific case, but the cooler is adjustable in at least two ways. In the first place the distance of the block from the end of the shoot can be changed within comparatively wide limits; in the second place the temperature of the air de-

livered may be further lowered by the addition of NaCl to the ice. Other salts can be used should a greater depression of the temperature be necessary.

The following table is the record of a test made at a room temperature of 87.8°F. The material in this particular case could

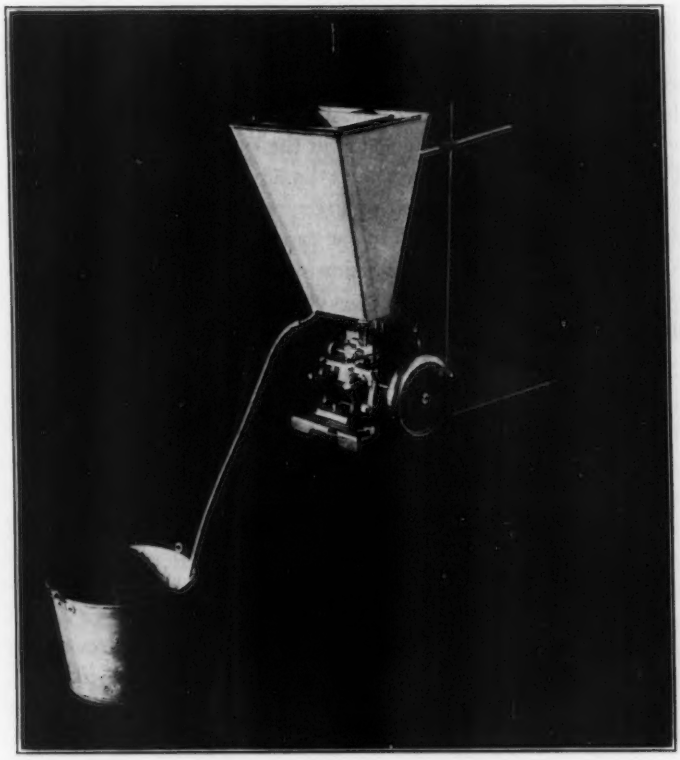


FIG. 1. Photograph of Cooler, by Miss Francis J. Dunbar. Measurements of pyramid: base, 12.5 in. x 8.7 in.; truncated apex, 6.1 in. x 2.1 in. Measurements of Ice-tray: 8.8 in. x 3.3 in. Cost, $3.50 without the standard.

not be imbedded in paraffin with high melting-point and satisfactory sections, even as thick as 12 micra, could not be cut. With the aid of the cooler however, a perfect series, 3 micra in thickness, was easily prepared from the same block of 45–48° paraffin.

TEST OF COOLER.

Room temperature .....31°C....87.8° F

Contents of Tray.	Distance below Mouth of Shoot.		
Crushed ice	6 cm.	24.5	76.1
Crushed ice + NaCl	6 "	23	73.4
Crushed ice	3 "	18	64.4
Crushed ice + NaCl	3 "	17	62.6

Several coolers, varying somewhat in size, but all modeled after our original one at Johns Hopkins University, are now in use in different laboratories. The measurements given in connection with Fig. 1, are those of the cooler at the University of Michigan. This particular one does not have the advantage of a removable ice-pan. In general, size is of little consequence unless it involves too great a reduction in the capacity of the ice-tray, or is conducive to too much absorption of heat by the sides of the pyramid. This latter difficulty is easily overcome by lining the shoot with asbestos paper.

JOHNS HOPKINS UNIVERSITY,
UNIVERSITY OF MICHIGAN.
July 15, 1910.

THE QUESTION OF REVERSAL OF ASYMMETRY IN THE REGENERATING CHELÆ OF CRUSTACEA.

CHARLES R. STOCKARD.

During the summer of 1909 while at the Tortugas Laboratory of the Carnegie Institution I undertook a further analysis of the reversal phenomenon in regenerating specimens of the genera *Alpheus* and *Synalpheus*. These small crustacea commonly called snapping shrimp, on account of their habit of snapping the large chelæ with such force as to produce a surprisingly loud noise, are abundantly found in the "logger-head" sponge and in the holes of disintegrating coral rock on the Tortugas reefs. There are a number of species five of which, *Alpheus formosus* and *armillatus* and *Synalpheus minus* and two other unidentified species of *Synalpheus*, were employed in these experiments.

The several species differ in size and body color but are essentially similar in general structure. The first pair of appendages is decidedly asymmetrical in both sexes. One member of the pair, either the right or the left, is extremely large, in some cases being more than half the size of the body itself. The general type of this chela in the five species follows more or less closely the description given by Wilson,[1] for the great chela of *Alpheus heterochelis*. It is greatly rounded or swollen with transverse grooves on either side of the propodus, varying in depth with the species, and presents characteristic color patterns being tipped with a lively rose color in *Synalpheus minus* while in the other species it is bluish, dark or brown. On the concave side of the dactylus is a swollen knob forming the "hammer" which fits into a corresponding socket on the outer side of the propodus claw. By extending the dactylus and then suddenly snapping the claws together the "hammer" is forced into the socket with the surprisingly loud sound.

Wilson's description further applies in that the large chela has essentially the same structure in both sexes, while the small

[1]Wilson, E. B., "Notes on the Reversal of Asymmetry in the Regeneration of the Chelæ in *Alpheus heterochelis*." BIOL. BULL., IV., pp. 197–210, 1903.

chela shows characteristic sexual differences, we shall be mainly concerned, however, with the fact that the small chela is always typically different from the large chela in shape as well as in size.

Przibram[1] discovered that in several species of *Alpheus* after the removal of the large chela a chela of the small type regenerated from its base while the small chela of the opposite side metamorphosed or developed into a great chela of typical form at the following moult. In other words, the asymmetry was reversed. Further, when both first chelæ are removed they regenerate in their original conditions, no reversal following.

Zeleny[2] found an exactly similar phenomenon to occur after removal of the functional operculum in the worm, *Hydroides*. In this case the rudimentary operculum of the opposite side develops into a functional operculum while a rudimentary organ regenerated from the base of the former functional one. The principle involved in this reversal phenomenon is doubtless the same as that in the crustacea.

Przibram[3] later found a similar reversal to occur in other species of crustacea, while in others the removal of either chela is followed by the regeneration of one of the simpler or smaller type without a regulatory change taking place in the uninjured chela of the other side. In still other cases, as for example the lobster, *Homorus*, a chela similar to the one removed invariably regenerates whether the original chela was a large crusher claw or the slender nipping claw.

The crustacea thus present a series from those forms which regenerate appendages of the type of the ones removed, others which regenerate appendages of the simpler type without a compensatory change taking place in the uninjured chela, and finally such forms as *Alpheus* in which the simpler type of chela is regenerated after the removal of the more specialized chela while the uninjured small chela develops into the more modified type,

[1]Przibram, H., "Experimentelle Studien uber Regeneration," *Arch. fur Entw.-Mech.*, XI., 1901.

[2]Zeleny, C., "A Case of Compensatory Regeneration in the Regeneration of *Hydroides dianthus*," *Arch. fur. Entw.-Mech.*, XIII., 4, 1902.

[3]Przibram, H., "Experimentelle Studien über Regeneration, II.," *Arch. für Entw.-Mech.*, XIII., 1901–1902; "Equilibrium of Animal Form," *Jour. Exp. Zoöl.*, V., p. 259, 1907–1908.

and thus by a sort of compensatory regulation the animal's asymmetrical condition is quickly reëstablished.

Wilson repeated Przibram's experiments on *Alpheus heterochelis* with similar results, but carried the experiments further, hoping to analyze the factors concerned in the reversal process. After removing the great chela the nerve trunk leading to the small chela of the opposite side was clipped in order to test whether there was a nervous control determining the growth of the small chela into a large one. After such an operation the small chela was generally thrown off and only two specimens are said to be beyond question, yet "one of these did not moult quite normally and the other not at all." The evidence, then, does not warrant conclusions as to the cause of reversal of asymmetry in the chelæ. Wilson finally believes that the initial factor that sets in motion the complex process of differentiation of which either side is capable, is primarily only a difference in the amount of material on the two sides. "Removal of the large chela obviously reversed the asymmetry in respect to the amount of material and must, temporarily, at least, lead to a functional nervous difference." Such a suggestion may easily be submitted to experimental test, for example, after removal of the large chela from one side of the body if several posterior appendages be removed from the other side the greater amount of material may still remain on the original large chela side. Fig. 1, *A*, illustrates the operation. Under these conditions will a large chela regenerate from the stump of the old one, instead of arising by a growth of the small first chela of the opposite side?

Again, the proposition may be tested by removing both the great and small chelæ of the first pair and in addition amputating several legs on the side of the large chela; the operation is illustrated by Fig. 1, *E*. The greater amount of material is now on the original small chela side; will this extra amount cause a great chela to regenerate from the small stump instead of from the stump of the great chela which is on the side with less material?

Lastly, when only a portion of the great chela is amputated does it regenerate in the original condition or become a small chela, while a large chela appears on the opposite side through a metamorphosis of the small first chela?

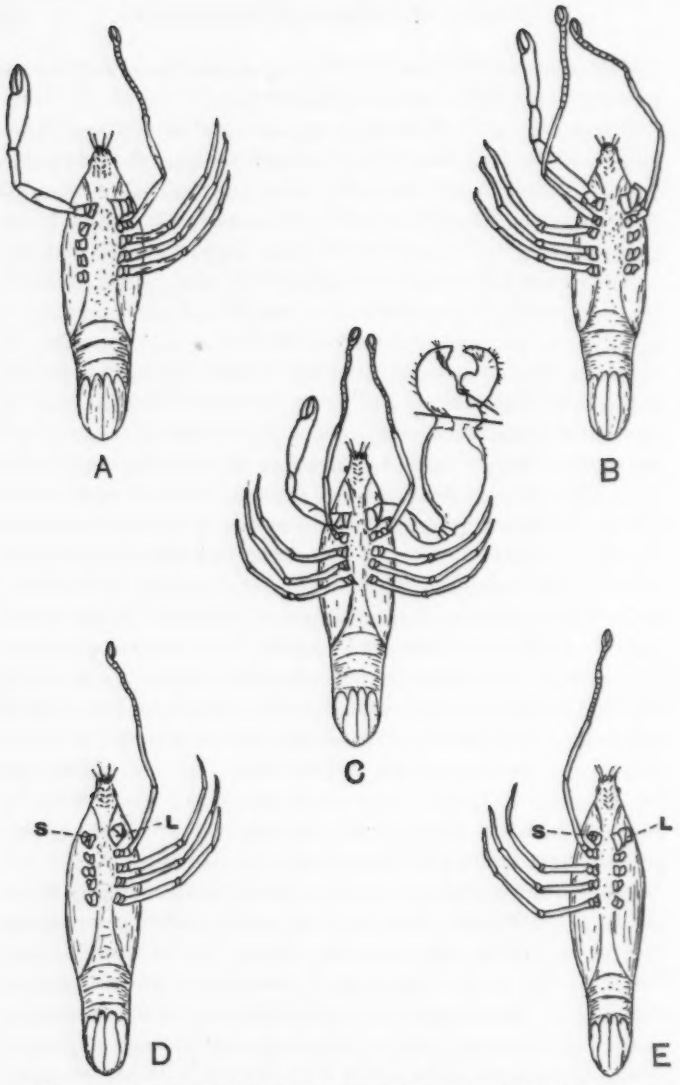


FIG. 1. Diagrams illustrating the manner of operation in the several experiments. *A*, removal of the great chela and the posterior appendages of the opposite side, causing the larger amount of material to still remain on the great chela side; *B*, the opposite operation as a control; *C*, the heavy lines show the places at which portions of the great chela may be cut away without reversal; *D* and *E*, removal of both first chelae and posterior appendages from either the small, *D*, or great, *E*, chela side, to determine the influence of the lateral amount of material on the regeneration of the first chelæ; *L*, large chela stump; *S*, small chela stump.

Aiming towards an answer for these questions a number of experiments were performed the results of which may now be considered.

Fifty healthy specimens representing the five different species were selected, and tested as to their tendency to reverse the asymmetry of the first pair of chelæ during the regeneration following the removal of the great chela. Without exception all of the specimens responded as Przibram had found, a small chela regenerated from the stump of the original great one and the small chela of the opposite side metamorphosed into a great chela. Forty of the specimens favorably survived the experiments.

First Series.

Nineteen individuals had the great chela removed and in addition a number of posterior appendages were amputated from the opposite or small chela side, so as to allow the greater mass of material to remain on the original great chela side. The operation is illustrated by Fig. 1, *A*, and the opposite or control operation by Fig. 1, *B*, or specimens 10, 12 and 31 in the table. Referring to the table the results of such an experiment may be ascertained.

The left side of the table gives the date of the operation, the number of the specimen, and the appendages removed are indicated by *x*, *G* and *S* in the first appendage column indicate the great and small chela. The right side of the table gives the time of moulting and the manner of regeneration, the *r* signifies a new or regenerated leg, *G* and *S* in the first appendage column again indicate the great and small chela. The horizontal lines of the table are so arranged that the appendages on the right side of an individual are given immediately above those on the left of the same animal, *e. g.*, specimen 1 had the great chela and the second leg removed from the right side and the fourth leg from the left side in the first instance. Each specimen, as the table shows, was operated upon a second time during the experiments.

Of the nineteen cases having the great chela removed from one side and other appendages than the first chela from the opposite side the small chela, even though it was on the side of less mate-

rial, retained the power to grow into a large chela of typical form in seventeen cases. One of these cases, specimen 23, is remarkable, since at the first operation the small chela which was on the left side and the other four left appendages were all removed and regenerated at the next moult. After this moult the large chela of the right side was amputated along with the four posterior legs of the left side, thus leaving only the regenerated small chela on the *left side* while the four posterior legs remained on the right side. Nevertheless, the left small chela grew into a great chela and the four more posterior left appendages regenerated for the second time. The case seems an extreme test of the power of one side to regenerate all of its posterior legs for a second time and in addition to change the first chela from the small to the large size and type. Specimen 25 further indicates this remarkable power of the side of the individual with less material to replace all lost parts and at the same time increase the size and type of its first chela.

The remaining two of the nineteen cases, specimens 9 and 18, present the first chelæ equal in size after the moult following the second operation. Specimen 9 had in the first case the great chela removed from the right side and the third, fourth and fifth appendages from the left side. The greater amount of material was, therefore, still on the right side, yet the small first chela of the left side became larger after the moult. The second operation removed the great left chela and the second, third, fourth and fifth legs of the right side. After the moult all of the amputated legs were regenerated but the regenerated left first was small, and the right first appendage had not increased in size. Thus the first pair were symmetrical in respect to size yet the right first or old chela had slightly approached the large chela type. The specimen 18 responded in a closely similar fashion.

The type of the chela is equally, if not more, important than the size since Przibram found the chelae to be of almost equal size in some cases but of reversed type, and the great type invariably increases in size at the following moult.

The experimental evidence in the first case, then, does not support the idea that the side with most appendage material

has most power to produce a great chela of typical size and form. The tendency is to produce a chela of the great size and type from the uninjured first chela, even though this be the original small chela and is located on the body side which has suffered the loss of all other walking appendages. There seems to be no evidence from these experiments to suggest a bilateral distribution of growth energy accompanying distribution of appendage material.

Second Series.

The question of a bilateral distribution of growth energy related to, or accompanying, the amount of appendage material on a given side was further tested in the following manner. Fifteen specimens were operated upon so as to remove both chelæ of the first pair. It was known that when only these two chelæ were removed that they regenerated in their original condition, a large chela from the base of the original large chela and a small chela from the opposite side. If now in addition to the removal of the first pair of appendages a number of more posterior appendages also be removed from the large chela side, this side will have less appendage material remaining and is, therefore, called upon to regenerate a greater amount of material to replace the posterior legs. Will this side of the body at the same time be more capable of producing a first chela of larger size and specialized type than the opposite side which is called upon to replace only the first chela? Such an operation is illustrated by Fig. 1, *E*, and Fig. 1, *D*, forms a control experiment in which the additional appendages are removed from the small chela side.

The usual idea of regulation would require the side with only the first leg removed to regenerate a large chela while the other side replaced the several posterior legs and produced a small first chela. It is found on examining the table that after an operation to remove both first chelae and one or more posterior legs on *either* the great or small chela side that six of fifteen specimens regenerated the chelæ of the first pair equal in size, not one individual reversed the type of the first chelæ, and eight regenerated the first chelæ in their original condition as though no additional appendages had been removed. One specimen died before the experiment was completed.

Considering the six specimens that regenerated the chelæ of the first pair equal in size it is important to find that four of these cases, specimens 16, 20, 21 and 39, had the larger number of posterior appendages removed from the side of the original small chela and not from that of the large chela, so that the greater amount of material remained on the large chela side. Such operations were intended as a control for the results following the removal of posterior appendages from the large chela side. Although in these four specimens there was more material on the original large chela side and this side was called upon to regenerate fewer appendages it failed to produce a great chela from the stump of the original one.

In specimen 16 the first chelæ remained equal in size and were both of the small chela type after a second moult. The left chela was then removed and after the next moult the right developed into a great chela and the left again regenerated small. Both first chelæ in specimens 20 and 21 were also of the small chela type, while in specimen 39 the small chela failed to regenerate at the first moult after the operation though a chela of the small type regenerated from the base of the great chela and remained small while the right small chela regenerated at the next moult.

Specimens 31 and 32 had both first chelæ and a number of appendages, four and three, on the great chela side removed. After the moult following the operation the first chelæ were equal in size. Yet again specimens 27, 35 and 38 were operated upon in an identical fashion and after the moult they were able to produce a great chela from the original great chela stump even though this side was called upon to regenerate three other appendages.

Of the fifteen cases tried, therefore, eight regenerate their first chelæ in the original condition of asymmetry while six regenerate the chelæ of the first pair equal in size and usually similar in type whether additional appendages are amputated from the great chela side or from the small chela side. Such a fact would seem to indicate that the amount of appendage material present on either side is an unimportant factor in determining the type of the first chela on a given side, and it seems to show further that there is no clearly evident bilateral distribution of growth energy in these regenerating specimens.

TABLE I.

THE EFFECT OF REGENERATION ON THE ASYMMETRICAL CONDITION OF THE FIRST PAIR OF CHELÆ IN FIVE SPECIES OF *Synalpheus* AND *Alpheus*.*

Date of Operation.	Specimen Number.		Appendages. 1	2	3	4	5	Date of Moult.	Appendages Regenerated. 1	2	3	4	5	Remarks.
May 24	1	R	Gx	x				June 4	Sr	r				
		L				x			G			r		
June 6	1	R		x	x	x		June 16	G		r	r	r	The small chela increases in size and changes its form to type of great chela.
		L	Gx						Sr					
May 24	2	R						June 1	G					
		L	Sx	x		x			Sr	r		r		
June 6	2	R	Gx	x				June 15	Sr	r				The third and fifth are only short buds, not fully regenerated.
		L		x	x		x		Gr	r	r		r	
May 24	3	R			x	x		May 30	G		r	r		
		L	Gx						Sr					
June 6	3	R	Gx	–				June 15	Gr	–				Only the dactyl removed and regenerated.
		L							S					
May 24	4	R	Gx					May 29	Sr					
		L							G					
June 6	4	R			x	x	x	June 13	G		r	r	r	Also moulted one day after the operation but had not regenerated
		L	Gx						Sr					
May 24	5	R						June 4	G					
		L	Gx						Sr					
June 6	5	R	Gx	x				June 13	Sr	r				Failure of third and fourth legs to regenerate might account for first chela becoming large.
		L		x	x	x	x		G	r	o	o	r	
May 24	6	R	Sx	x	x	x		June 1	Sr	r	r	r		
		L	Gx						Gr					
June 6	6	R		x	x	x	x	June 12	G	r	r	r	r	
		L	Gx						Sr					
May 24	7	R						June 3	G					Moulted few hours after operation on May 24 and right first chela began to increase in size.
		L	Gx		x				Sr		r			
June 6	7	R	Gx					June 13	Sr					
		L			x	x	x		G		r	r	r	
May 24	8	R	Gx					May 30	Sr					
		L							G					
June 7	8	R				x	x	June 14	G			r	r	Also moulted two days after operation but no regeneration.
		L	Gx						Sr					
May 24	9	R	Gx					May 29	Sr					Moult followed so soon after operation that left first chela had not attained full size, but did so after a few days.
		L			x	x	x		G		r	r	r	
June 7	9	R		x	x	x	x	June 14	S	r	r	r	r	Also moulted 2 days after operation. Right first somewhat similar to great chela type but small.
		L	Gx						Sr					
May 24	10	R						May 30	G					
		L	Gx	x		x	x		Sr	r		r	r	

TABLE I.—*Continued.*

Date of Operation	Specimen Number.		Appendages. 1	2	5	4	5	Date of Moult.	Appendages Regenerated. 1	2	3	4	5	Remarks.
June 7	10	R	Gx	x				June 14	Sr	r				Operated immediately after a moult on June 7.
		L				x	x		G			r	r	
May 24	11	R			x	x		May 30	G		r	r		
		L	Gx						Sr					
June 7	11	R	Gx	x				June 13	Sr	r				
		L			x	x	x		G		r	r	r	
May 24	12	R	Gx					June 2	Sr					
		L		x	x	x			G	r	r	r		
June 7	12	R						June 15	G					
		L	Gx		x	x	x		Sr		r	r	r	
May 24	13	R	Gx				x	May 29	Sr				r	
		L					x		G				r	
June 7	13	R												Died after operation.
		L	Gx											
May 24	14	R	Gx	–			x	June 1	Gr				o	Only the dactylus and index cut from great chela, no reversal.
		L												
June 7	14	R	Gx	–				June 14	Gr					Second operation and result the same as first.
		L												
May 24	15	R	Gx					June 3	Gr					
		L	Sx						Sr					
June 7	15	R	Gx	x				June 15	Sr	r				
		L			x		x		G		r		r	
May 24	16	R	Gx				x	May 31	Sr				r	After a second moult on June 7 the first chelae were still of equal size.
		L	Sx			x	x		Sr			r	r	
June 7	16	R						June 13	G					The left first chela was removed and right became large and left regenerated small.
		L	Ex						Sr					
May 25	18	R			x			June 1	G		r			
		L	Gx						Sr					
June 7	18	R	Gx					June 14	Sr					Pincer of left first seems slightly nearer the great chela type.
		L			x	x	x		S		r	r	r	
May 25	19	R	Gx					June 3	Sr					
		L							G					
June 8	19	R	Sx		x			June 15	Sr		r			
		L	Gx			x	x		Gr			r	r	
May 25	20	R			x	x	x	June 6	G		r	r	r	
		L	Gx						Sr					
June 8	20	R	Gx					June 19	Sr					Both first chelae of the small type and equal in size.
		L	Sx			x	x		Sr			r	r	
May 25	21	R		x		x	x	May 30	G	r		r	r	
		L	Gx						Sr					
June 8	21	R	Gx	x				June 13	Sr	r				Both first chelae of equal size and small type.
		L	Sx	x		x			Sr	r		r		
May 25	23	R		x		x		June 4	G	r		r		
		L	Sx	x	x	x	x		Sr	r	r	r	r	
June 7	23	R	Gx					June 15	Sr					Second regeneration of all posterior left legs, yet left first became large chela.
		L		x	x	x	x		G	r	r	r	r	
May 25	25	R			x	x	x	June 1	G		r	r	r	
		L	Gx						Sr					

TABLE I.—*Continued.*

Date of Operation.	Specimen Number.		Appendages. 1	2	3	4	5	Date of Moult.	Appendages Regenerated. 1	2	3	4	5	Remarks.
June 10	25	R	Gx		x	x	x	June 17	Gr		r	r	r	Again second regeneration on one side yet first chela of this side becomes large.
		L	Sx			x			Sr			r		
May 25	26	R						June 6	G					
		L	Gx		x				Sr		r			
June 10	26	R	Gx											Did not moult the second time.
		L				x	x							
May 25	27	R		x	x	x		June 4	G	r	r	r		
		L	Gx			x			Sr			r		
June 10	27	R	Gx		x	x	x	June 17	Gr		r	r	r	
		L	Sx						Sr					
May 25	28	R	Gx				x	June 2	Sr				r	
		L							G					
June 8	28	R		x	x	x	x	June 14	G	r	r	r	r	Second pair of legs regenerated shorter than usual.
		L	Gx	x					Sr	r				
May 25	29	R	Gx					June 6	Sr					Moulted day before the operation.
		L							G					
June 8	29	R			x	x	x	June 16	G		r	r	r	
		L	Gx						Sr					
May 25	30	R					x	May 29	G-				r	Moult followed so soon after operation that little increase in right chela, no regeneration of left, after second moult June 7 condition complete
		L	Gx	x					o	r				
June 10	30	R	Gx		x	x	x							Died June 16 without moulting.
		L	Sx											
May 25	31	R						June 3	G					
		L	Gx	x	x		x		Sr	r	r		r	
June 10	31	R	Gx	x	x	x	x	June 18	Sr	r	r	r	r	First chelæ equal in size and of small type.
		L	Sx						Sr					
May 25	32	R			x	x	x	May 31	G		r	r	r	
		L	Gx						Sr					
June 10	32	R	Gx		x	x	x	June 15	Sr		r	r	r	Both first chelæ smaller than normal but of equal size.
		L	Sx						Sr					
May 25	33	R		x	x	x		May 30	G	r	r	r		
		L	Gx						Sr					
June 10	33	R	Gx	x	x	x		June 16	Gr	r	o	o		Failure of 3 and 4 to regenerate may account for first chela's growth.
		L	Sx						Sr					
May 25	35	R	Gx					May 29	Sr					
		L			x				G		r			
June 8	35	R	Sx					June 14	Sr					Left first chela great though regenerating three other legs on same side.
		L	Gx	x	x		x		Gr	r	r		r	
May 25	36	R	Gx					June 3	Sr					Also moulted 2 days after operation.
		L			x	x			G		r	r		
June 8	36	R			x	x		June 15	G		r	r		
		L	Gx						Sr					
May 25	37	R						May 30	G					Moulted again June 6.
		L	Gx			x			Sr			r		
June 8	37	R	Gx		x	x	x	June 15	Gr		r	r	r	
		L	Sx						Sr					

TABLE I.—*Continued.*

Date of Operation.	Specimen Number.		Appendages. 1	2	3	4	5	Date of Moult.	Appendages Regenerated. 1	2	3	4	5	Remarks.
May 25	38	R						May 30	G					
		L	Gx			x	x		Sr			r	r	
June 8	38	R	Gx	x	x	x		June 15	Gr	r	r	r		Also moulted June 9 day after operation.
		L	Sx						Sr					
May 25	39	R	Sx	x	x			June 6	o	r	r			
		L	Gx			x			Sr			r		
	39	R						June 14	Sr	r	r			First chelæ finally regenerated equal in size and remained so after next moult
		L							Sr			r		
May 25	40	R	Sx					May 30	Sr					Moulted again June 6.
		L	Gx			x	x		Gr			r	r	
June 8	40	R	Sx			x	x	June 15	Sr			r	r	
		L	Gx						Gr					

Each specimen was operated upon twice as indicated. R and L following the specimen number signifies right and left sides of the animal; G indicates the great and S the small first chela; x indicates the appendages removed and r the appendages regenerated.

Third Series.

Finally, an attempt was made to determine how large a portion of the great first chela might be removed without causing it to regenerate small; or to cause the small chela of the opposite side to grow into the great type. When a large portion of the chela was quickly clipped off with sharp scissors or a knife the remaining portion was soon thrown off at the breaking joint. The only successful operations consisted in the removal of the dactylus or most distal segment which forms part of the claw, and in the removal of the entire pincer or dactylus and distal end of the propodus, as is indicated by the lines drawn across the chela in Fig. 1, *C*. In the last case a stump-like appendage without a pincer remains.

Following either of these operations the great chela was fully reformed or renewed at the next moult, no reversal taking place.

A small portion of the great chela may then be regenerated in its original form. When the entire chela is removed the small chela of the opposite side invariably grows into a great chela and a small chela regenerates from the stump of the original great one. This reversal of asymmetry may be shifted back and forth for a number of times and occurs in a manner as decidedly pronounced after several operations as it does after the first.

Conclusions.

The power to reverse the asymmetry of the first chelæ when regenerating a great claw in *Alpheus* does not seem to be closely associated with a difference in the amount of material on the two sides of the body nor with a bilateral distribution of growth or regenerative energy.

Although in certain cases there seems to be a tendency to regenerate the chelæ of the first pair equal in size and similar in type, such a tendency is manifest under conditions so varied in respect to the bilateral distribution of appendage material and call upon the powers of regenerative energy that the present conclusion is warranted. The amount of material on a given side of the animal, or the amount of regeneration required of this side are negative factors in determining the ability of the side to produce a great chela instead of a small one.

Naples, July 5, 1910.